REMARKS

Claims 44-91 are pending in the application. All stand rejected under 35 USC § 103 for obviousness.

Rejections under 35 USC § 103

Claims 68-91 were rejected under 35 USC § 103(a) as obvious over US Patent No. 6,673,575 (*hereinafter,* Franze). The Office Action states that Franze suggests the use of fructose, galactose, and mannose and also the use of sugars in different combinations. The Action states that "no patentable invention resides in combining old ingredients of known desired function where the results obtained thereby are no more than the additive effect of the ingredients." The Action says that it was a matter of routine experimentation to combine fructose, mannose, and galactose for use in culture media to control sialylation. Finally, the Action states, "The depth of the prior art is significant and clearly has established that the selection of sugar, amounts thereof and other normal culture parameters are result effective variables."

A *prima facie* case of obviousness requires (1) the disclosure of all claim elements in one or more prior art references, (2) a suggestion to modify or combine the references to achieve the claimed invention, and (3) a reasonable expectation of success. MPEP § 2143. Here, a reasonable expectation of success is lacking because, as explained below, it would not be reasonable to expect success based on the disclosure of Franze. Thus, since a proper *prima facie* case has not been made, claims 68-91 are not obvious.

Applicant submits herewith a declaration under 37 CFR § 1.132 of Dr. Carole Heath, Dr. Heath's Curriculum Vitae, and a translation of an Experimental Report (included in the Informational Disclosure Statement submitted herewith), which was originally submitted by Kirin Brewery Co. Ltd. in its appeal of Japanese Patent No. 3394240 B2 (the Japanese Patent corresponding to US 6,673,575, which was invalidated at trial in Japan) and a translation of which was submitted by Amgen Inc. in an opposition to European Patent No. 1 036 179 (the European Patent corresponding to US Patent No. 6,673,575).

Applicant submits that it is not possible to unambiguously interpret the experiments described Examples 5 and 7 of Franze. Franze contains no experimental support outside of Examples 5 and 7 for the proposition that feeding a culture with

multiple sugars increases protein glycosylation above that observed in a culture fed with glucose without other sugars. Therefore, if Examples 5 and 7 do not provide experimental support for this proposition, there is none. In the attached declaration, Dr. Heath states that potentially important experimental factors are not clearly specified in Examples 5 and 7 and that, therefore, one of skill in the art could not unambiguously conclude which experimental factor(s) influenced the outcomes of these experiments. She enumerates a number of factors that might be responsible for the observed results, only one of which is that the cultures were fed with multiple sugars instead of glucose without other sugars. In essence, the declaration states that the controls, as described in Franze, do not make it possible to determine which experimental factor(s) is/are responsible for the outcome of these experiments. Thus, Franze provides one of skill in the art with no reasonable basis for concluding that the addition of other sugars (specifically, galactose and mannose), along with glucose, to a culture medium would increase protein glycosylation over that observed when glucose is added without other sugars.

Kirin's Experiment Report (submitted with the attached Information Disclosure statement) discloses an attempt to duplicate some of the experiments of Franze. In order to do this, the Kirin scientists necessarily had to resolve ambiguities in the Franze disclosure, which they did as follows: "when the experimental conditions were not clearly stated in the specification of this patent, typical experimental conditions were followed." Experimental Report, p. 5. Kirin's results showed no clear difference in the distribution of isoforms of erythropoietin produced by cultures fed with a nutrient solution containing glucose as compared to cultures fed with a nutrient solution containing either (1) glucose and mannose, (2) glucose and galactose, or (3) glucose, galactose, and mannose. Experimental Report, Figure 1, p. 7, Tables 1 and 2, pp. 8-9, and p. 10. These data indicate that feeding with any of these combinations of sugars has essentially no effect on protein sialylation when a culture fed with glucose without other sugars is used as a control. This is consistent with Dr. Heath's description of the expectations of one of skill in the art based on the disclosure of Franze because one of skill in the art would have considered a number of explanations for the Franze results other than the explanation that feeding with multiple sugars caused increased sialylation. Thus, these data suggest that one of the

other possible explanations may be the correct explanation of the results described in Examples 5 and 7 in Franze.

Dr. Heath further states that one of skill in the art would not consider all monosaccharides and disaccharides, even the eleven named sugars (Franze, col. 3, lines 4-9), to be interchangeable with respect to their effects on protein glycosylation. She asserts that different sugars interact with different enzymes, a fact that would lead one of skill in the art to believe that not all sugars will behave similarly. She points to a publication (Stark and Heath (1979), Arch. Biochem. Biophys. 192(2):599-609, which is of record) that demonstrates that a number of sugars do not, in fact, behave similarly with respect to their effects on protein glycosylation. Thus, even if, for the sake of argument, one of skill in the art were to believe, that the experimental design of Examples 5 and 7 of Franze is adequate to show that feeding with glucose, mannose, and galactose generally increases glycosylation relative to that observed when feeding glucose, such a person of skill would still have no reason to believe that other, untested, combinations of sugars would have similar effects. Thus, based on the disclosure of Franze and knowledge in the art, one of skill in the art would not have had a reasonable expectation at the filing date of the instant application that feeding a mammalian cell culture producing a protein with combinations of sugars other than glucose, mannose, and galactose would increase protein glycosylation.

As an additional ground for nonobviousness, Applicant's representative submits that the selection of a species from the huge genus disclosed by Franze is nonobvious under cases such as *In re Baird* (16 F.3d 380, 29 USPQ2d 1550 (Fed. Cir. 1994)). The invention of Franze is described as a process for isolating a glycosylated polypeptide from a culture of eukaryotic cells, cultured in a medium to which at least two or three carbohydrates or at least one carbohydrate and/or at least one essential amino acid is/are added. *See* Franze col. 2-3, lines 64-3, claims 1 and 4. The genus of carbohydrates that can be used to form the combinations of at least one, two, or three carbohydrates is described as follows:

> monosaccharides and disaccharides such as glucose, glucosamine, ribose, fructose, galactose, mannose, sucrose, lactose, mannose-1-phosphate, mannose-1-sulfate and mannose-6-sulfate.

Franze, col. 3, lines 4-8. The specific combinations of claims 68-91, that is, 1) fructose and galactose and 2) mannose, fructose, and galactose, are not mentioned in Franze. Patents are relevant for all they contain, not only selected portions. *In re Lemelson,* 158 USPQ 275, 277 (CCPA 1968). Thus, the disclosed genus is **not** limited only to combinations containing one or more of the eleven named carbohydrates, which is already a large number. (As stated in the submission mailed April 10, 2006, Applicant's representative believes that this number is 2047.) Applicants submit that 2047 is a large genus and that it is not obvious to select a specific combination from this large genus. However, the disclosed genus also includes **all monosaccharides and disaccharides and all combinations thereof.** This is clearly a very large genus, given the huge number of compounds that would qualify as monosaccharides and disaccharides.

For the sake of getting an idea of the numbers involved, Applicant's representative submits a non-exhaustive list of commercially available monosaccharides and disaccharides, which includes the following: D-allose, D-(-)-arabinose, α-D-glucose, 2-deoxy-D-glucose, 6-deoxy-D-glucose, 2-deoxyribose 5-phosphate, D-mannitol, D-(-)-erythrose, D-(-)-fructose, D-(+)-fucose, D-(+)-galactose, 3-α-galactobiose, β-gentiobiose, D-glucoheptose, D-sorbitol, N-acetyl-D-galactosamine, N-acetylallolactosamine, isomaltose, kojibiose, lacto-N-biose, α-lactose, lactulose, α-3-fucosyl-N-acetylglucosamine, D-(-)-lyxose, D-(+)-maltose, N-acetyl-D-lactosamine, D-(+)-arabitol, 2α-mannobiose, palatinose, melibiose, D-psicose, L-rhamnose, D-(-)-ribose, D-ribulose, sophorose, D-(+)-sorbose, sucrose, D-(-)-tagatose, D-(+)-talose, D-(-)-threose, α,β-trehalose, 2,5-anhydro-D-mannitol, and D-(+)-xylose. These were found in the Sigma-Aldrich catalog available at http://www.sigmaaldrich.com/Area_of_Interest/The_Americas/United_States.html. Applicant does not suggest by providing this list that these are the only existing monosaccharides and disaccharides or that the disclosure of Franze is limited to commercially available monosaccharides and disaccharides. Looking at groups containing 1 to 43 of these 43 monosaccharides and disaccharides, Applicant's representative calculates that about 8.8×10^{12} different groups could be formed.[1] This number is an underestimate of the total possible number of combinations of all

[1] The formula *n!/k!(n-k)!* describes the number of different groupings of *n* different objects in group sizes containing *k* objects can be formed. Grossman and Turner, pp. 24-30 (of record).

monosaccharides and disaccharides, since the starting list contains only a subset of all monosaccharides and disaccharides. Still, this underestimate qualifies as a large genus under cases such as *In re Baird (supra)* in which the large genus in question contained only 100 million (10^8) according to the estimate of the court. *Baird, supra,* at 382. Therefore, Applicant's representative submits that the selection of any individual combination from this huge genus is nonobvious. MPEP § 2144.08; *In re Baird, supra.* Moreover, even the somewhat smaller genus (2047) of all groups of one or more of the eleven named sugars is also huge from the point of view of a researcher trying to determine which, if any, of these combinations are effective in controlling glycosylation. If the Examiner does not agree that the size of the genus is sufficient to make the selection of a species therefrom nonobvious, Applicant requests clarification on this issue.

The USPTO offers guidance as to how to assess obviousness in cases where a single prior art reference is found to disclose a genus encompassing a claimed species. MPEP §2144.08. A *prima facie* case must first be established under the *Graham* factors. *Graham v. John Deere,* 383 U.S. 1 (1966). This requires that "Office personnel find some motivation or suggestion to make the claimed invention in light of the prior art teachings." MPEP §2144.08 (II)(A). "In order to find such motivation or suggestion there should be a reasonable likelihood that the claimed invention would have the properties disclosed by the prior art teachings." MPEP §2144.08 (II)(A). The MPEP presents a decision tree to aid in making this decision. The considerations include the following: 1) Is the genus so small that each member is inherently disclosed? 2) Are there express teachings that would have motivated the selections? 3) Is there a teaching of structural similarity? and 4) Is there any other teaching to support the selection of the species or subgenus? An answer of "No" to each of these questions leads to a determination of nonbobviousness. MPEP §2144.08 (III).

Applicant asserts that the answer to each of the above-mentioned questions is "No" and that the claims are therefore nonobvious. First, as discussed above, Franze discloses a huge genus of all combinations of monosaccharides and disaccharides. Therefore, the genus is not so small that each member is inherently disclosed. If the Examiner does not agree that this is the case, Applicant respectfully asks for clarification as to the basis of the Examiner's position. Second, there are no express

teachings in any cited reference that would have motivated the selection. Third, although Franze may teach that all combinations of one or more monosaccharides and/or disaccharides increase glycosylation, the Kirin experimental report shows that this simply is not the case, even for the one case that was actually tested in Franze. Moreover, the Kirin report, according to Dr. Heath's declaration, is consistent with what one of skill in the art could reasonably conclude based on the disclosure of Franze. In addition, Dr. Heath points out that different sugars would be expected to interact with different enzymes, and, thus, all sugars would not be expected to have the same effects on glycosylation. Stark and Heath (*supra)* demonstrate that this expectation is, in fact, true. Thus, there is no credible teaching of structural similarity. Finally, there is no other teaching in the art to suggest the selection of the claimed species. Thus, claims 68-91 are nonobvious. If the Examiner does not agree, Applicants respectfully request clarification with respect to how an answer of "yes" could be given to any of the three questions recited above.

In the event that the Examiner should still maintain that one of skill in the art would have a reasonable expectation of success and that a *prima facie* case of obviousness has therefore been made, Applicant argues in the alternative that unexpected results sufficient to rebut the *prima facie* case have been shown. If one were to take all assertions in Franze at face value, any monosaccharide or disaccharide added to a culture medium should increase protein glycosylation relative to that observed when a culture is grown in glucose without other sugars added. The instant specification shows that unexpectedly, based on the Franze disclosure, some combinations do not increase protein sialylation and that some do. Thus, unexpected results sufficient to rebut obviousness are shown.

With reference to claims 68-91, the instant specification shows that addition of either fructose or mannose alone has no effect sialylation relative to that observed with standard, glucose-containing medium, while addition of galactose increases sialylation. Figure 2. Addition of the combination of galactose and fructose to a standard medium increases protein sialylation more than addition of galactose alone, which is a greater than additive effect because fructose alone has no effect on sialylation. Figure 2. Combinations of either mannose and fructose or mannose and galactose decrease sialyation relative to standard medium. Figure 2. However, the combination of fructose, mannose, and galactose increases sialylation, to only a

slightly lesser degree than the combination fructose and galactose. Figure 2. Thus, the combination of fructose, mannose, and galactose also displays non-additive, unexpected effects when compared to the combinations of galactose or fructose plus mannose. Thus, the differences observed in the instant specification between different combinations of carbohydrates is non-additive and unexpected over the disclosure of Franze, which posits that any monosaccharide or disaccharide or any combination thereof can be used to increase protein glycosylation.

The Office Action also rejects claims 44-67 as obvious over Franze in view of Schnaar et al., Wood, Gu et al., or Gu et al. The Action states that Gu, Wood, and Schnaar teach the controlling of sialylation of protein by exposing cells to N-acetylmannosamine and that Franze suggests that combinations of sugars are beneficial. Claims 44-67 are directed towards media and methods for its use, wherein the medium comprises either 1) mannose, fructose, galactose, and N-acetylmannosamine or 2) galactose and N-acetylmannosamine.

One of skill in the art could not reasonably expect to be successful at increasing protein glycosylation by selecting one or more sugars disclosed in Franze and combining these with N-acetylmannosamine. First, as explained above, one of skill in the art would not have a reasonable expectation that the selection of one or more of the sugars disclosed in Franze would increase glycosylation because such an increase was not demonstrated to be due to addition of multiple sugars in even the one tested case in Franze (see the Kirin report and the declaration of Dr. Heath). Further, one of skill in the art would have no reasonable basis for expecting that any particular, untested monosaccharide and/or disaccharide and/or combinations thereof disclosed in Franze would increase protein glycosylation or that all combinations of monosaccharides and/or disaccharides would behave similarly with respect to protein glycosylation. *See* Dr. Heath's declaration. Moreover, also as explained above, the selection of a single combination of sugars from the huge genus described in Franze is not obvious. In addition, although the work of Gu and Wang (Biotechnol. Bioeng. 1998; 58:642-48, which is of record) indicated that addition of N-acetylmannosamine to culture medium could increase protein glycosylation, other researchers have not found this to be always true. *See* Baker et al. (2001), Biotechnol. Bioeng. 73:188-202, at paragraph spanning pp. 196-197; Hills et al. (2001), Biotechnol. Bioeng. 75:239-61, at abstract, p. 244, left column, last paragraph, and p. 249, left column. Such

"teach away" references are highly relevant to a determination of obviousness, and they point to a lack of a reasonable expectation of success in using the addition N-acetylmannosamine to medium to increase protein glycosylation. If the Examiner does not agree, Applicant requests clarification on this issue.

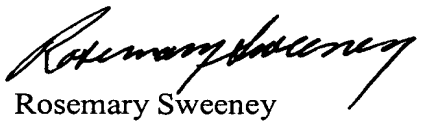
Alternatively, should it be deemed that a *prima facie* case of obviousness has been made, Applicant asserts that unexpected results sufficient to overcome obviousness are shown in the instant specification. If one were to take all assertions in Franze at face value, any monosaccharide or disaccharide added to a culture medium should increase protein glycosylation relative to that observed when a culture is grown in glucose without other sugars added. The instant specification shows that unexpectedly, based on the Franze disclosure, some combinations do not increase protein sialylation and that some do. Thus, unexpected results sufficient to overcome obviousness are shown. Specifically with reference to claims 44-67, addition of either fructose or mannose plus N-acetylmannosamine decreases sialylation relative to that observed with standard, glucose-containing medium. Figure 2. Addition of galactose and N-acetylmannosamine to a standard medium increases protein sialylation relative to that observed when the standard medium is used alone or when fructose or mannose plus N-acetylmannosamine is added. Figure 2. Thus, it is an unexpected result that different combinations of sugars behave differently. Further, in light of these results, it is additionally surprising that the addition of mannose, galactose, fructose, and N-acetylmannosamine increases sialylation over that observed when only galactose and N-acetylmannosamine have been added. This effect is more than additive since the combinations of fructose or mannose plus N-acetylmannosamine have negative, not positive, effects on glycosylation. Figure 2. These results are non-additive and unexpected based on the disclosure of Franze, which suggests that any monosaccharide or disaccharide or combination thereof can be used to practice the invention. For this reason and all those reasons discussed above, Applicant submits that claims 44-67 are not obvious.

Conclusion

Applicant believes that all claims are in condition for allowance and respectfully requests notice to that effect. Should the Examiner believe that any

remaining issues can be resolved most easily via teleconference, he is invited to telephone the undersigned at the direct dial number listed below.

Respectfully submitted



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Attachment:
Declaration Under 37 CFR 1.132

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